LETTER TO THE EDITOR

On the Interpretation of X-Ray Diffraction Intensities from Chemically Treated Frog Sciatic Nerve

Dear Sir:

In the February issue of this journal, Akers and Parsons (1970) describe X-ray data from frog sciatic nerve after treatment with heavy atoms and they try to account for the observed intensities by assuming that gaussian distributions of heavy atoms accumulate at $x = 0$ and $x \approx d/2$. This location of heavy atoms is claimed to support the choice of all positive phases for the first five diffraction orders of frog sciatic nerve. I find this interpretation of the X-ray intensities from chemically treated nerve to be unconvincing and consequently I believe that the choice of all positive phases is unsound.

Akers and Parsons (1970) argue that it is reasonable to assume an accumulation of heavy atoms at $x = 0$ and at $x \approx d/2$ as suggested by electron microscopy. Now in this location the heavy atoms have positive phases and, because the first five X-ray intensities show increases after chemical treatment, it is claimed that the first five diffraction orders of untreated nerve also have positive phases. In order to examine the correctness of this claim, there are two important questions to consider. The first question asks whether or not it is reasonable to assume that the distribution of heavy atoms in chemically treated nerve is the same as that shown by nerve when examined by electron microscopy. The second question asks whether or not this gaussian distribution at $x = 0$ and $x \approx d/2$ together with the choice of all positive phases for untreated nerve does, in fact, provide an explanation of the observed intensities.

In some respects it is reasonable to expect that the location of heavy atoms in chemically treated nerve to be closely related to the dense and intraperiod lines shown by nerve in electron micrographs. However, electron microscopy cannot answer the first question because only nerves which have undergone the specimen preparation procedures of electron microscopy have been examined. On the other hand X-ray diffraction does provide information on this question. X-ray patterns have been recorded from nerves treated in the same way as the electron microscopy nerve specimens (Fernandez-Moran and Finean, 1957). These X-ray patterns are markedly different from the X-ray patterns of chemically treated nerve and this indicates that the transformation of chemically stained nerve to nerve treated for electron microscopy involves additional structural changes. Hence, the idea that the heavy atoms in chemically treated nerve have the same location as shown by nerve in electron micrographs is not directly supported by X-ray diffraction.

In order to answer the second question a set of corrected intensities $J(h)$ is needed rather than the usual set of observed intensities $I(h)$ according to Worthington and Blaurock (1969 *a*). Akers and Parsons (1970) give X-ray intensities from nerve treated using osmium tetroxide vapour and platinic chloride and, surprisingly, these two sets of X-ray intensities at labeling time $t = 0$ are different! These X-ray intensities all increase initially with uptake of heavy atoms. From diffraction theory, if the gaussian distribution model was correct and, if all positive phases are assumed for untreated frog sciatic nerve, then $\partial J(h)/\partial t$, the slope of the intensity increase with time, has the following variation: (2) > (4) > (3) > (5) > (1).

However, in the case of osmium tetroxide vapour the slopes are seen to have the following magnitudes: (2) > (5) $\approx$ (3) > (4) > (1) and this disagreement indicates that the Akers and Parsons (1970) model does not give good agreement with the observed intensities recorded from nerve treated with osmium.

Patterson functions of frog sciatic nerve chemically treated with mercuric chloride and treated with osmium have been described by Millington and Finean (1958) and by Akers and Parsons (1970), respectively. These Patterson functions were computed using $I(h)$ instead of the corrected intensities $J(h)$ and are therefore strictly incorrect although the principal features are still apparent. The Patterson function of live nerve shows a strong peak at $d/2$ and a minor peak at $d/4$ (Worthington and Blaurock, 1969 *a*). The Patterson functions of the two different chemically treated nerves are similar in the case of the 60 mg mercury per dry weight and in the case of the 15 min labeling time for osmium; they both show a strong peak at $x \approx 68$ A but the minor peak at $d/4$ is missing.

Now, for argument's sake, if the second question is reconsidered and now the question asked is whether or not the accumulation of heavy (osmium) atoms at $x = 0$ and $x \approx d/2$ can provide an explanation of the Patterson function of chemically treated nerve. This location of heavy atoms gives rise to a strong osmium-osmium correlation and the resulting peaks in the Patterson are centered at $x = 0$ and $x = d/2$. Now the Akers and Parsons (1970) intraperiod gaussian distributions centered at $x = 0.45d$ and $0.55d$ would provide a single peak at $x = d/2$ in the Patterson function and this leaves the strong peak at $x \approx 68$ A unexplained and the missing $d/4$ peak is also unexplained. Therefore, the Akers and Parsons (1970) model does not provide an explanation of the observed Patterson function of chemically treated nerve.

In an earlier study the relationship between certain features of the Patterson function and parameters of membrane-type structures has been established (Worthington, 1969 *a*). This leads to a straightforward interpretation of the Patterson function of chemically treated nerve. In live nerve the membrane pair thickness is 156 A (Worthington, 1969 *b*) and the resolved Patterson peak of $x \approx 68$ A suggests a reduced membrane pair thickness of about 140 A for chemically treated nerve. In terms of an electron density model for live nerve (Worthington, 1969 *b*), if the heavy atoms enter the cytoplasmic and extracellular regions and are dispersed throughout the high electron density regions but, enter only sparingly, if at all, the low density regions, then the X-ray intensities of the first five orders all show increases. The variation of intensities between diffraction orders will depend on the actual shrinkage of the membrane pair. This model for chemically treated nerve accounts for the experimental facts and provides an explanation of the observed Patterson function.

The phase problem of swollen nerve (nerve swollen in hypotonic solutions) has been solved (Worthington and Blaurock, 1969 *a*). The adoption of this phase solution to live nerve provides the choice of $(-++--)$ phases for the first five diffraction orders although the phase of the first order is not known as well as the other four phases. Note, this choice of phases was first used in an earlier study by Finean and Burge (1963). The reasons why the phases for live nerve can be obtained from swollen nerve have been given elsewhere (Worthington and Blaurock, 1969 *a*). For the present purposes it is noted that the transformation of live nerve to swollen nerve is reversible and although the molecular structure of swollen nerve differs from that of live nerve, these differences are quite small. This transformation has been studied by X-ray diffraction (Worthington and Blaurock, 1969 *b*) and the observed X-ray data are consistent with the choice of $(-++--)$ phases for live nerve. The proposed model for chemically treated nerve also has the same set of phases as live nerve.

In summary, Akers and Parsons (1970) interpretation of the X-ray intensities of chemically treated nerve does not fit the experimental facts and the subsequent choice of all positive

phases is untenable. An alternative model is proposed which provides an explanation of the observed Patterson function of chemically treated nerve. Hence, the choice of phases ($-++--$) for nerve myelin remains valid.

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REFERENCES

AKERS, C. K., and D. F. PARSONS. 1970. *Biophys. J.* **10**:116.
FERNANDEZ-MORAN, H., and J. B. FINEAN. 1957. *J. Biophys. Biochem. Cytol.* **3**:725.
FINEAN, J. B., and R. E. BURGE. 1963. *J. Mol. Biol.* **7**:672.
MILLINGTON, P. F., and J. B. FINEAN. 1958. *J. Ultrastruct. Res.* **2**:215.
WORTHINGTON, C. R. 1969 *a*. *Biophys. J.* **9**:222.
WORTHINGTON, C. R. 1969 *b*. *Proc. Nat. Acad. Sci. U.S.A.* **63**:604.
WORTHINGTON, C. R., and A. E. BLAUROCK. 1969 *a*. *Biophys. J.* **9**:970.
WORTHINGTON, C. R., and A. E. BLAUROCK. 1969 *b*. *Biochim. Biophys. Acta.* **173**:427.

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